



A Review on Solvent Use in HPLC

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ABSTRACT:

High-Performance Liquid Chromatography (HPLC) is one of the newest and accurate analytical techniques used for the various things like separation, identification, and quantification of complex mixtures in pharmaceuticals, biotechnology, and environmental sciences. The efficiency and reproducibility of HPLC highly depend on the solvent system employed in the mobile phase. Solvents play a main role in determining the selectivity, resolution, retention time, and peak symmetry of analytes. The choice of solvents is influenced by some factors such as polarity, viscosity, UV transparency, miscibility, and pH stability. The classification of HPLC solvents can broadly be divided into aqueous and organic components. Aqueous solvents, including pure or buffered water, provide stability and polarity control, while organic modifiers like methanol, acetonitrile, and tetrahydrofuran enhance elution strength and selectivity. One of the most sophisticated and accurate analytical methods for the separation, identification, and measurement of complex mixtures in the fields of environmental sciences, biotechnology, and pharmaceuticals is High-Performance Liquid Chromatography (HPLC). The solvent system used in the mobile phase has a significant impact on HPLC's efficiency and repeatability. The selectivity, resolution, retention time, and peak symmetry of analytes are all significantly influenced by solvents. Numerous factors, including polarity, viscosity, UV transparency, miscibility, and pH stability, affect the choice of solvents. Aqueous and organic components make up the two main categories into which HPLC solvents are classified. Organic modifiers such as methanol, acetonitrile, and tetrahydrofuran improve elution strength and selectivity, while aqueous solvents, such as pure or buffered water, offer stability and polarity control.

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is a versatile analytical tool widely used for separating and quantifying compounds in pharmaceutical formulations, food products, and biological samples (1). Its effectiveness depends significantly on the choice of mobile phase solvents, which govern analyte solubility, interaction with the stationary phase, and overall chromatographic performance (2). The mobile phase in HPLC typically consists of one or more solvents that facilitate the elution of analytes through the stationary column under high pressure (3). Solvent composition, polarity, and pH directly influence retention time, selectivity, and peak shape (4). For instance, methanol and acetonitrile are commonly used organic solvents due to their low UV cut-off, excellent miscibility with water, and chemical stability (5). Solvent purity is equally crucial, as impurities can introduce background noise and baseline drift during UV or fluorescence detection (6). Degassing and filtration steps are employed to remove dissolved gases and particulate matter that may interfere with pressure stability or detector response (7). Additionally, environmental and health concerns have encouraged the exploration of less toxic, bio-derived solvents to replace hazardous ones such as tetrahydrofuran (THF) and chloroform (8). The understanding of solvent interactions—both with analyte and immobilized phases—is essential for optimizing chromatographic efficiency. Solvent parameters like dielectric constant, overview moment, and viscosity help tell in advance chromatographic behavior under specific conditions (9). Consequently, solvent choice is not merely a procedural step but a scientifically strategic decision that determines the analytical success of HPLC request (10).

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2. Classification of Solvents Used in HPLC:

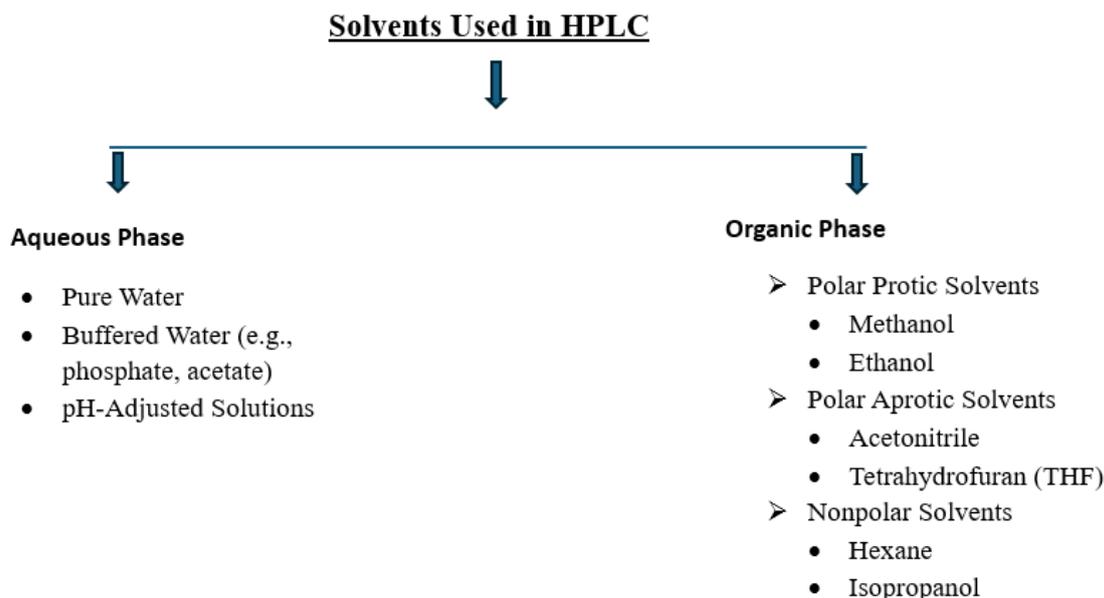


Fig:1 Solvents Used in HPLC (1,11,13)

Aqueous Phase:

Water is the primary solvent in the aqueous phase due to its polarity and ability to dissolve a wide range of ionic and polar compounds (11). Buffered systems, such as phosphate or acetate buffers, maintain a stable pH environment, which is vital for ionizable analytes (12). pH-adjusted solutions prevent changes in analyte charge states and improve retention consistency (13).

Organic Phase:

Organic solvents are incorporated to control elution strength and polarity balance. Polar protic solvents like methanol and ethanol form hydrogen bonds and are suitable for polar analytes (14). Polar aprotic solvents such as acetonitrile and THF provide higher elution strength with reduced viscosity (15). Nonpolar solvents like hexane and isopropanol are mainly used in normal-phase HPLC where hydrophobic interactions dominate (16).

4. Criteria for Selecting Solvents in HPLC

Choosing an appropriate solvent is essential for method reliability and reproducibility (17). Parameters such as polarity, viscosity, and UV transparency significantly affect chromatographic performance (18). Low-viscosity solvents (e.g., acetonitrile) decrease column backpressure and enhance resolution (19). Solvents must be UV-transparent within the detector range (190–254 nm) to avoid interference (20). Miscibility between organic and aqueous components is necessary to maintain homogeneous phases during gradient elution (21). pH stability ensures that ionizable analytes remain in their desired form, while chemical purity minimizes baseline noise (22). The chosen solvent should also exhibit minimal reactivity with analytes and stationary phase materials (23).

5. Role of Solvents in Different HPLC Modes

The function of solvents varies depending on the chromatographic mode employed. In reverse-phase HPLC (RP-HPLC), nonpolar stationary phases (e.g., C18) interact with hydrophobic analytes, while the mobile phase is more polar (24). Increasing the proportion of organic solvent decreases analyte retention time. In normal-phase HPLC, polar stationary phases require less polar solvents like hexane or isopropanol for effective separation (25). In ion-exchange HPLC, solvents maintain ionic strength and pH to regulate electrostatic interactions between charged analytes and the stationary phase (26). Size-exclusion chromatography utilizes aqueous or organic solvents that prevent analyte–stationary phase interaction and ensure molecular sieving (27).

6. Solvent Effects on Chromatographic Parameters

Solvents influence retention time, selectivity, resolution, and peak symmetry. The polarity index of the solvent affects analyte–stationary phase affinity, while viscosity influences diffusion and mass transfer rates (28). The solvent's refractive index and dielectric constant also impact detector response and peak shape (29). Proper solvent selection reduces tailing, improves sensitivity, and ensures reproducible chromatograms (30).

7. Solvent Preparation and Degassing Methods

Proper solvent preparation ensures consistent of various chromatographic conditions. Filtration through 0.45 μm membranes removes most of the particulates, avoiding blockages in columns or pumps (31). Degassing eliminates dissolved gases that can cause bubble formation and detector noise. Common techniques which used

vacuum filtration, helium sparging, and ultrasonic degassing (32). Solvents should be freshly prepared and stored in amber bottles to prevent photodegradation (33).

8. Environmental and Safety Considerations

Many traditional organic solvents create health and environmental hazards. Acetonitrile and THF, though efficient, are toxic and also flammable (34). Green chemistry encourages the use of bio-based solvents (e.g., ethanol, propylene carbonate) to minimize toxicity and waste of solvent (35). Proper solvent disposal and recovery systems help to reduce laboratory pollution and operational costs (36). Laboratories are increasingly trying solvent recycling systems and reduced-flow HPLC systems for sustainable operation (37).

9. Recent Advances and Trends

Recent research focuses on eco-friendly solvents like ionic liquids, supercritical CO₂, and deep eutectic solvents, which offer various polarity and minimal environmental impact (38). Advances in micro-HPLC and UHPLC have further reduced solvent consumption while enhancing separation efficiency (39). The development of solvent-free or solvent-minimized extraction and analysis methods reflects a increasing trend toward sustainable analytical practices (40).

10. Conclusion

Solvents form the backbone of HPLC performance, dictating separation efficiency, reproducibility, and analytical precision. Their correct selection and management are central to achieving optimal chromatographic outcomes. The choice of solvents must balance several parameters, including polarity, viscosity, UV transparency, and miscibility, all of which influence resolution and retention behaviour. With increasing emphasis on environmental responsibility, the field has progressed toward green and sustainable solvent systems. Ethanol, ionic liquids, and supercritical fluids represent promising alternatives options to conventional organic solvents, offering comparable chromatographic efficiency with reduced toxicity. Advances in UHPLC technology have reduced solvent use without compromising with analytical quality, aligning laboratory practices with modern sustainability goals. Based on environmental aspects, safety, cost, and compatibility with various detection systems remain critical considerations. Proper solvent handling, degassing, and waste management reduce instrumental wear and analytical error. Continuous innovation in solvent technology—combined with automation and miniaturization—promises to redefine HPLC efficiency in the future.

In conclusion, solvent optimization is not merely a preparatory step but a fundamental analytical decision that determines method robustness, reliability, and reproducibility. As HPLC continues to evolve, the future lies in integrating performance-driven solvent selection with eco-conscious methodologies, ensuring both scientific excellence and environmental stewardship in analytical chemistry.

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